

Serial No. 09/091,605
Amendment dated September 22, 2003
Reply to Office action of April 21, 2003

Specification Amendments

Please amend the specification by inserting the replacement paragraphs presented below in which the changes are shown by strikethrough or underlining. The amendments are corrections of misspellings and do not add new matter.

PAGE 9:

To effect the expression of a ~~polypeptides~~ polypeptide of SEQ ID NO 1, one ligates a nucleotide sequence encoding the polypeptide into an appropriate recombinant nucleotide expression vector through the use of appropriate enzymes.

The nucleotide sequence encoding a polypeptide of SEQ ID NO 1 is designed to possess restriction endonuclease cleavage sites at either end of the DNA to facilitate isolation from and integration into these amplification and expression plasmids. The coding sequence may be readily modified by the use of synthetic linkers to facilitate the incorporation of the coding sequence into the desired cloning vectors by techniques well known in the art. The particular endonucleases employed will be dictated by the restriction endonuclease cleavage pattern of the parent expression vector to be employed. The choice of restriction sites are chosen so as to properly orient the coding sequence such that it is properly associated with the promoter and ribosome binding site of the expression vector, both of which are functional in the host cell in which a compound of SEQ ID NO 1 is to be expressed.

PAGE 9 - 10

In addition to the desired ~~nucleotide~~ nucleotide sequence which will code for the therapeutic protein, the expression vector may contain several other functional elements. One such element is the promoter and upstream regulatory sequences which control the level of expression of the protein of interest. Some expression vectors contain promoters and regulatory sequences which normally regulate transcription of cellular genes. One such promoter is the mouse metallothionein-I promoter which has been shown to function both in-vitro and in-vivo (Palmiter et al., Nature 300: 611-615, 1982). In addition, promoters and regulatory sequences from viruses are frequently used in expression vectors (Dijkema et al., EMBO J. 4, 471, 1985; Gorman et al., Proc. Natl. Acad. Sci. 79: 6777, 1982; Boshart, et al. Cell 41:521, 1985). Although, Verma et al. have shown that the retrovirus promoter and enhancer sequences do not function for long periods of time in-vivo. In addition, the expression vector also carries an origin of replication as well as marker sequences which are capable of providing phenotypic selection in transformed cells.

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PAGES 10-11

Stable transformed cell lines that express proteins of SEQ ID NO 1 must then be implanted into the individual in need of such treatment. Because such transformed cell lines generally will be histologically incompatible with the b individuals receiving them, the cells must be protected from the recipient's immune system. ~~Once~~ One way of protecting the implanted cells is by masking them with F(ab')₂ fragments specific for HLA class I antigens. Immunological masking methods are well known in the art. For example, see Faust et al. Science 252: 1700-1702 (1991). Other means for protecting the implanted cells from the recipient's immune system are consistent with this invention. Such methods include but are not limited to encapsulation in semi-permeable membranes (Lanza et al. Diabetes 41: 1503 - 1510) and through the use of immunosuppressants (Rynasiewicz et al., Diabetes 31: 92 -108, 1982).